

DE NOVO SEQUENCING AND TRANSCRIPTOME ANALYSIS OF *KALANCHOE* TO IDENTIFY PUTATIVE GENES INVOLVED IN EPIPHILLY

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In nature, asexual reproduction plays an important adaptive role. The process is widespread to several families (e.g. *Crassulaceae*, *Graminaceae*, *Liliaceae*). Among *Crassulaceae*, vegetative vivipary (epiphilly) has been reported on leaves, stems and flower stalks of many *Kalanchoe* species. In *K. xhoughtonii*, a tetraploid interspecific hybrid, viviparous plantlets are formed on leaf margin notches in response to a long day photoperiod and their appearance follow a basipetal fashion.

Recently, next generation sequencing (NGS) technologies provide new options to understand complex phenomena in non-model species developing affordable tools for functional genomics.

With the purpose of identify and characterize genes putatively involved in vegetative vivipary and in leaf morphogenesis, *de novo* sequencing and analysis of *K. xhoughtonii* transcriptome were performed by 454 pyrosequencing technology.

Total RNA was isolated from apical meristems (comprehensive of leaf primordia) and from leaf at diverse stages of development (0,5 , 1 and 3 cm), using RNeasy Plant Mini kit (QIAGEN) to prepare two full length cDNA libraries (labelled M_ and S_Library), respectively. Library normalization and pyrosequencing with the GS FLX 454 Titanium system was performed by Eurofins MWG-Operon (Ebersberg, Germany).

454 reads from the two libraries were independently assembled using MIRA Assembler. The 578.856 and 593.130 HQ reads obtained with two half-plate run from the M_library and S_Library were assembled in 120.650 and 138.816 unigenes (contigs plus singletons), respectively. The unigene sequences were annotated by BLASTx versus non-redundant protein database (nr) with Blast2GO bioinformatics tool (<http://www.blast2go.org>). In both libraries, the species that provided most of the top BLAST hits was *Vitis Vinifera* (about 25.600 and 23.061 genes, respectively).

The ORFPredictor tool (<http://proteomics.ysu.edu/tools/OrfPredictor.html>) : computed that about 99,5% contigs in both libraries correspond to protein-coding genes.

The Gene Ontology annotation and “Augment Annotation by ANNEX” function, to refine annotations, was done using Blast2GO. 54,08% and 57,6% contigs were annotated in the S_ of, and the M_library, respectively. The GO terms obtained by the annotation procedure were mapped to a plant specific GO-Slim to simplify and highlight differentially expressed GO classes. KEGG orthologs and biological pathways were assigned to unigenes.

Overall, differences were displayed among GO classes between the two libraries. The GO terms analysis was resulted 836 and 854 genes in the transcription factor activity (level 3 of molecular function) of M and S_Library, respectively. In particular putative genes and gene family involved in vivipary and in leaf morphogenesis were identified.